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Effect Of Prior Use Of Desensitizing Agents On Bond Strength Of Adhesive Systems To Human Dentin

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ABSTRACT

The aim of this study was to evaluate the effect of desensitizing agents on the bond strength of different adhesive systems condition and rinse and self-etching to dentin through microshear. Sixty dentin fragments were used, obtained from the buccal and lingual surfaces of molars third molars, which were distributed in groups (n = 12) according to desensitizing treatment (control - no treatment; potassium oxalate; + CPP-ACP fluorine; arginine; nano-hydroxyapatite) and adhesive system employ (Single Bond Adpter 2 / 3M; Clearfil SE Bond / Curaray). The applications of desensitizing agents (2 ml) were performed in three sessions with an interval of 48 hours. The dentin fragments remained immersed in 6 ml of artificial saliva in the period between applications. The adhesive systems were applied according to the instructions of the manufacturer and cured for 40 seconds. About every two dentin surfaces were fabricated in composite cylinders. The mechanical test was performed using a microshear speed of 1 mm / min .The fracture patterns were determined using a digital microscope with increased 50 times. Fractures were classified as adhesive, cohesive in dentin and composite resin cohesive described percentage. Data were compared by 2-way ANOVA and the Bonferroni test ($p < 0.05$).
It was concluded that the desensitizing treatments influenced the bond strength of adhesive systems and conditional wash; treatment with potassium oxalate and arginine reduced the bond strength of self-etching; treatment with CPP-ACP did not interfere with hydroxyapatite and adhesion of self-etching.

Keywords: Erosion. Dental Enamel. Desensitizing; microshear

1. Introduction

The increase in life expectancy of human beings and their association to numerous preventive measures including reducing loss of dental elements for caries and periodontal disease, occurred at the same time a significant increase in the incidence of other problems in the oral cavity as those arising from the dental wear and dentin sensitivity.

Current studies show that more than 30% of adults have Dentinal hypersensitivity. It is a localized pain in one or more teeth that is exacerbates by thermal stimuli, evaporative, tactile, osmotic, chemical or, especially when there are already pathological defect. This pain occurs when there is stimulation on Dentinal tubules, which happens because the osmotic exchanges of fluid is present within the Dentinal tubules.

The hydrodynamic theory of classical Bra̎nnsto̍rm, which showed the movement of fluid inside the tubules that excite the nerves, is the cause of the pain directly linked to the intensity of this movement.

Desensitizing agents act to occlude these tubules open and protect them from any kind of recurrence, depending on its effectiveness.

However, there are numerous questions by topic dental erosion and its interaction with bioactive compounds in the form of toothpastes or other products with benefits and the full compatibility of its use without interference on adhesion of human dentin adhesive system.

The aim of this work is to evaluate the effect of the prior application of different desensitizing agents (control - without treatment; potassium oxalate; CPP-ACP + fluoride; arginine; nano-hydroxyapatite) on Union resistance of adhesive systems and adhesive systems employ (Adpter Single Bond 2/3M; Clearfil SE Bond/Curraray) to human dentin.

2. Proposition

Evaluate the effect of different desensitizing agents prior application (control-without treatment; potassium oxalate; CPP-ACP + fluoride; arginine; nanohidroxiapatita) on bond strength of adhesive systems and adhesive systems employ

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(2/3 m Single Bond Adpter; Clearfil SE Bond/Curraray) to human dentin.

3. Material and Methods

3.1 Experimental Design

See Figure 1 (A,B,C,D,E) - representative schematic Image of the experimental design

3.2 Preparation of the Specimens

30 healthy molars were selected that remained stored in distilled water until the beginning of the experimental phase. The dental elements received cuts crosswise in the middle third of the root using a disc (Extec, Enfield, CT, USA) with the aid of a metallographic leafcutter (Labcut, model 1010, Extec, Enfield, CT, USA) and received cuts serials towards ocluso-cervical in order to obtain two dentin fragments, from the vestibular and lingual surfaces figure 1 A/B). The fragments were stored in vials identified.

3.3 Desensitizing Treatment

Dentin fragments except those intended for the control group were exposed to 2 ml of desensitizing agents, for 15 min.

The technical specifications as trademark, chemical composition of desensitizing agents and application protocols can be observed in table 1.

See table 1 - Showing trademark, chemical composition and application protocol.

The applications of desensitizing agents were held in three sessions with an interval of 48 hours. The fragments remained immersed in 6 ml artificial saliva whose composition (potassium chloride-0.96g, sodium chloride-0.67g, magnesium chloride-0.04g, potassium phosphate-0.27g, calcium chloride-0.12g, nipagin-0.001g, nipasol-0.1 g, methyl cellulose carboxyl group-8g, solbitol-24g and purified water qsp (1000 ml), in the period (seven days) between the applications. The saliva was replaced daily.

After treatment with the desensitizing agents was made the adhesive area delimitation was carried out with double sided adhesive tape acid-resistant to micro-shear test. Cut tape fragments were large enough to cover half the surface of each specimen. Each tape fragment received two circular holes lined with 1 mm in diameter made with a rubber sheet punch. A sticky back tape was adhered to the fragment, bordering two dentin surfaces with a diameter of 1 mm, on which were applied the adhesive systems according to the respective manufacturers ' instructions (Shimaoka, 2010).

Of two dentin fragments obtained from the same dental element and treated with the same desensitizing agent, one received the adhesive procedure with the condition system and wash and the other fragment received the adhesive with the self-etching system procedure.

Were fixed on the dentin surface previously delimited with adhesive tape a Silicon matrix (d-1 mm; 1.5 mm-h), which was filled with composite (Z250 - 3 m/ESPE) with the aid of a capacitor, and light-cured with the curing light (Elipar Freelight 2 LED --3 m ESPE) by 40 seconds.

Before light curing of each experimental group measured employing a light LED radiometer-Demetron.

Then, the array was cut with a razor (Haine) and removed. The specimens were stored in artificial saliva for 15 min in organic greenhouse (Orion, Fanen SP Brazil), to 37°C.

See table 2- adhesive systems evaluated, chemical composition and application protocol

Mechanical testing of micro-shear was performed as described by Shimaoka *et al*. (2011). The dental fragments were fixed to the device of universal testing machine (INSTRON 5942 CT, USA) based adhesive materials of cyanoacrylate so that the composite cylinders remain aligned to load cell.

A metallic wire of 0.2 mm in diameter lassoes the prolongation of machine and load cell simultaneously on each composite cylinder. The wire was kept in contact with the lower semi-circle of cylinders, maintaining contact with dentin surface (Figure 1 E).

A shear force was applied with the speed of 1 mm/min until the fracture moment of the specimen. The results expressed in Newtons films in Mpa.

After testing the fracture pattern, micro-shear of each sample was determined with the aid of a digital microscope with increased 50 times (MiView USB Digital Microscope, Chinavasion Wholessale, Guandong, CN).

The fractures were classified as adhesives (Interfacial failures in substrate and adhesive), dentin, cohesive cohesive in resin (composite resin and or failures in the layer of adhesive) and mixed (more than one of the types cited) and described in proportion.

4. Results

The data obtained through the mechanical test of micro-shear and expressed in MPa were submitted to analysis of variance (ANOVA 2-way) for two factors of variation (adhesive and dentin treatment system). The comparison between the groups for evaluation of statistical differences was performed by Bonferroni test. All testing employ admitted as statistical significance level of 5%.

The averages and the respective standard deviations of experimental groups for the different experimental conditions are expressed in table 3.

See table 3-test results micro-shear relating to mechanical treatments desensitizers and adhesive systems.

The Bonferroni test showed that the results of micro-shear obtained by groups without treatment in etch adhesive system and wash differs from the other treatments desensitizers.

In the treatments with potassium oxalate, arginine and nano-hidroxiapatite, did not differ among themselves for the etch adhesive system and wash.

Treatment with CPP-ACP presented the results micro-shear higher when compared to other types of desensitizing treatment for the condition system and wash.

The treatments with CPP-ACP and nano-hydroxyapatite did not differ from the control group. However the group with potassium oxalate differed from the control group, taking arginine shown the smallest results in terms of adhesion to the self-etching adhesive system. In the control group to etch and rinse and auto-conditioned there were no differences among themselves.

The treatments with potassium oxalate and the CPP-ACP and the control group did not differ among themselves.

The statistical analysis showed that treatment with potassium oxalate and CPP-ACP and the control group did not show statistical differences between both adhesive systems evaluated.

However the treatment with arginine showed significantly higher results of micro-shear when compared to the self etch adhesive system.

The results obtained with the nano-hidroxiapatite showed higher values and statistically different between the self-etching primer adhesive system in relation to etch adhesive system and wash.

And it is important to stress that all treatments desensitizers influenced on the results obtained in the etch adhesive system and wash.

Potassium oxalate, arginine and nano-hidroxiapatite influenced negatively by reducing the adhesion when system condition and wash are used.

The CPP-ACP favored the adhesion when used with the condition system and wash.

This treatment also did not influence on adhesion to dentin the self-etching adhesive.

See Figure 2 - results in MPa, desensitizing agents control groups and the two self-etching adhesives and wash and condition.

The percentage distribution of the types of fracture can be seen in Chart 1.

See Chart 1 - Percentage of the types of fractures of desensitizing substances control group and the stickers self-etching primer and etch and rinse.

The experiment conducted in the control group, when it was used the self-etching adhesive showed result of four types of fracture being a percentage equal to the adhesive and cohesive interfaces into substrate, outnumbered the mixed, and a very low percentage the cohesive in composite resins.

When the adhesive has been used condition and wash there was predominance of mixed fractures and a little below the adhesive interface fractures, there is neither a kind of cohesive fractures in substrate and cohesive in composite resin.

In the Group of potassium oxalate, there was representation from the four types of fractures, mainly in a high percentage of mixed fractures especially when self-etching adhesive was used, which predominated in high percentage on cohesive fractures in substrate, with the adhesive and cohesive interface interface in composite resin in low percentage.

The same thing happened when adhesive etch and rinse were used with a predominance of mixed with the cohesive fractures in substrate and cohesive in matching composite resin in percentage with few fractures of adhesive interface.

When we used the desensitizing, CPP-ACP using the auto adhesive etching predominated the percentage of mixed fractures compared the adhesive interface and to a lesser percentage the cohesive in composite resin.

When the adhesive has been used condition and wash the percentage of mixed fractures predominated in greater numbers than when we used the predominating in large percentage of self-etching the adhesive interface and fractures in low percentage to be cohesive in composite resin.

On the other hand when it was used as a desensitizing the arginine in conjunction with self adhesive condition there was a large percentage of adhesive interface fractures on the mixed, having a very low percentage of cohesive fractures in composite resin.

Already in use of arginine in conjunction with etch adhesive and wash the percentage of mixed fractures surpassed the percentage of cohesive in composite resin and the adhesive interface.

The use of Nano P desensitize (FGM), with nanoparticles technology of calcium and phosphate that confers bioactivity to dental materials.

Chemical and structural characteristics as the resemblance to natural hydroxyapatite of teeth, having even greater biocompatibility, remineralizing, dimensional nanometric, greater bioactivity.

Its mechanism of action is the deposition of hydroxyapatite in the Dentinal tubules, forming a waterproof film on the dentin, hindering access to Dentinal tubules.

When in this study nanohidroxiapatite were used with a self-etching adhesive percentage between the adhesive interface fractures and joint fractures. When used with the adhesive condition and wash there was a percentage between the adhesive interface fractures and fractures mixed and slightly less cohesive fractures in composite resin.

5. Discussion

The results of the treatments performed with the potassium oxalate, arginine and nano-hidroxiapatite did not differ among themselves for the condition system and wash.

The treatment effected with CPP-ACP presented higher micro-shear results when compared to other types of desensitizing treatment for the condition system and wash.

When using the treatments with CPP-ACP and nano-hidroxiapatite not occur differences between control group. However the group with potassium oxalate that difference happened, namely arginine showed lower results in terms of adhesion to the self-etching adhesive system. In the control group to etch and rinse and self-etching no difference between them. Statistical analysis showed that treatment with potassium oxalate, CPP-ACP and the control group did not show statistical differences between both adhesive systems evaluated. However, when using the treatment with arginine results were significantly higher than micro-shear when compared to the self etch adhesive system.

The results obtained with the use of nano-hidroxiapatite showed higher values and statistically different between the self-etching primer adhesive system and the etch adhesive system and wash.

It is important to state that all treatments desensitizers influenced on the results obtained in the etch adhesive system and wash.

In this context, the potassium oxalate, arginine and nano-hidroxiapatite influenced negatively, i.e. promoted the reduction of the membership when it used the etch adhesive system and wash. Concordant with present research, Borges (2012), investigated, in vitro, the effect of applying a folder containing casein phosphopeptide -amorphous calcium phosphate (CPP-ACP) (MI Paste-MI) before the Union resistance adhesive Protocol of a pit and fissure sealant resin to tooth enamel 98 specimens of human proximal enamel of third molars, concluding that the enamel contact with CPP-ACP prior to application of adhesive systems was an effective method to increase endurance of Union between enamel and adhesive.

In another job with results similar to the work of Porto et al. (2012), examined the effect of potassium oxalate dentin exposed to 35% phosphoric acid, followed by Scotchbond Multipurpose adhesive systems and Prime Bond, followed by the application of composite resin Z100. The control group did not receive the application of oxalate, and two groups received the oxalate, getting the three groups in distilled water at 37°C for 12:0 am and during 12 months. Were submitted to micro-shear test results was very similar to those of the groups of this research.

Trushkowsky and Oquendo (2011), presented similar results to the current research to conduct a study with the potassium oxalate dentin resulting in calcium oxalate crystals which occluded the Dentinal tubules, and interfered with resin/dentin adhesion. The authors recommend avoiding the application of adhesives acididicos, as this may compromised the adhesion.

Silva *et al*. (2010), in their study suggest that the combination of the potassium oxalate with the acid attack of the adhesives on dentin/resin adhesion interfere when used adhesive condition and wash, being therefore, compliant with the present work. However, the same authors use the patches of two or three steps (Single Bond, one-step and Scotchbond respectively, when tested immediately 12:0 am and after 12 months stored in water) did not interfered on dentin resin adhesion, using potassium oxalate demonstrating a discordant results.

When the CPP-ACP the same favored membership in association with etch adhesive system and wash. However, this treatment also did not influence on adhesion to dentin the self-etching adhesive, i.e. was the compound used that showed better results.

Adebayo *et al*. (2008) in his work "CPP-ACP Complex as a new adjunctive agent for remineralisation, concluded that there was no significant effect on the micro-shear test when used the self-etching adhesives as well as etch and rinse. CPP-ACP treatment did not affect the 2 adhesives and self-etching nor steps prior to treatment, according to the current work.

In the survey of Gupta and Prakash (2011) the same presented the mechanism of action of CPP-ACP as incipient caries preventive, as remineralizing agent and obviously as desensitizer of enamel and dentin, the application of CPP-ACP can influence on adhesion to dentin, dissenting from the current research where the referenced component was the least influence on adhesion to dentin.

Another result of concordant present research was the work of Garcia *et al*. (2010), that when using arginine to 8% associated with fluoride and calcium carbonate no influences on dentin enamel membership subject to the micro-shear test.

In the work of Silva (2012) named "Influence of applying desensitizers on the adhesive strength of the findings of two adhesives" the author is comparing the percentage of spontaneous fracture during the mechanical test using desensitizing substances, however still no conclusive results.

It was observed during the bibliographical research of the work a lack of search engine results that are nearing completion, making it impossible for the comparison of the results with these works.

As regards the comparison of the percentage of the types of fractures the experiment performed in the control group, with the self-etching adhesive showed the result of four types of fracture with a percentage equal to the cohesive and adhesive interface in substrate, being outnumbered mixed fractures, and in a minimum percentage the cohesive in composite resin. However, when using the adhesive condition and wash there was a predominance of mixed fractures and to a lesser percentage of the adhesive interface fractures, not any kind of cohesive fractures in composite resin substrate and cohesive.

When using the Group of potassium oxalate in the representation of the four types of fractures, occurred the predominance of mixed fractures, especially when used the self-etching adhesive. However, when using the adhesive condition and wash in the same group, also occurred the predominance of mixed with the cohesive fractures in substrate and cohesive in matching composite resin in percentage and with few fractures type adhesive interface. At work use clinical evaluation of potassium oxalate in restorations of non-carious cervical lesions de Souza (2011), the author analyzed the efficiency of use of potassium oxalate in restorations of non-carious cervical lesions, the author concluded that the employment of the potassium oxalate dentin pretreatment agent did not influence the clinical performance of the restorations of non-carious cervical lesions at the end of a year, concordant with this research.

The use of potassium oxalate dentin desensitizer reduces as the permeability and adhesion to dentin according to the article de Silva *et al*. (2010) also concordant with the results of the present research.

As to the percentage distribution of the types of fracture compared to CPP-ACP desensitising when applied the adhesive self etching mixed type fractures predominated over the adhesive interface and these fractures compared cohesive type fractures in composite resin. When the adhesive condition and wash was used the percentage of mixed fractures predominated in greater number than when using the self-etching adhesive, predominantly in large percentage on the adhesive interface and the fractures in composite cohesive. In Borges's work (2010), the author investigated the in vitro effect of application of a folder containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) prior to the Union resistance adhesive Protocol of a pit and fissure sealant resin to human dental enamel, concluding that the enamel contact with CPP-ACP prior to application of adhesive systems is an effective method to increase the bond strength between enamel and pit and fissure sealants, being similar to that of the present research.

Therefore, when using arginine, in research with the self-etching adhesive there was a large percentage of adhesive interface type fractures in relation to mixed, and a minimum percentage of cohesive fractures in composite resin. Even when etch adhesive was used and wash the percentages of mixed fractures exceeded the cohesive in composite resin and the adhesive interface.

Godoy (2010) in his work "a desensitizing paste effect on Union of dentin adhesive system" concluded that arginine 8% did not alter the adhesion to human dentin, contrary to the results for the same substance during the present research.

When nano-hidroxiapatite was used with the self-etching adhesive occurred a percentage between the adhesive interface fractures and joint fractures. To be applied with adhesive condition and wash resulted in a percentage between the adhesive interface fractures and fractures mixed and slightly less cohesive fractures in composite resin.

In another interesting article the author reported that beyond the kind of dentin, superficial or deep, the adhesives used, and the length of time in storage can affect the bond strength to the material used, as well as the storage time factor can influence.

6. Conclusions

Desensitizing treatments used influenced on bond strength of adhesive systems etch and rinse.

Treatment with potassium oxalate and arginine has reduced the bond strength of self-etching primer adhesive systems.

Treatment with CPP-ACP has not interfered in the adhesion hydroxyapatite of self-etching primer adhesive systems.

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Appendix

Figure 1 (A,B,C,D,E): Schematic image representative of experimental design

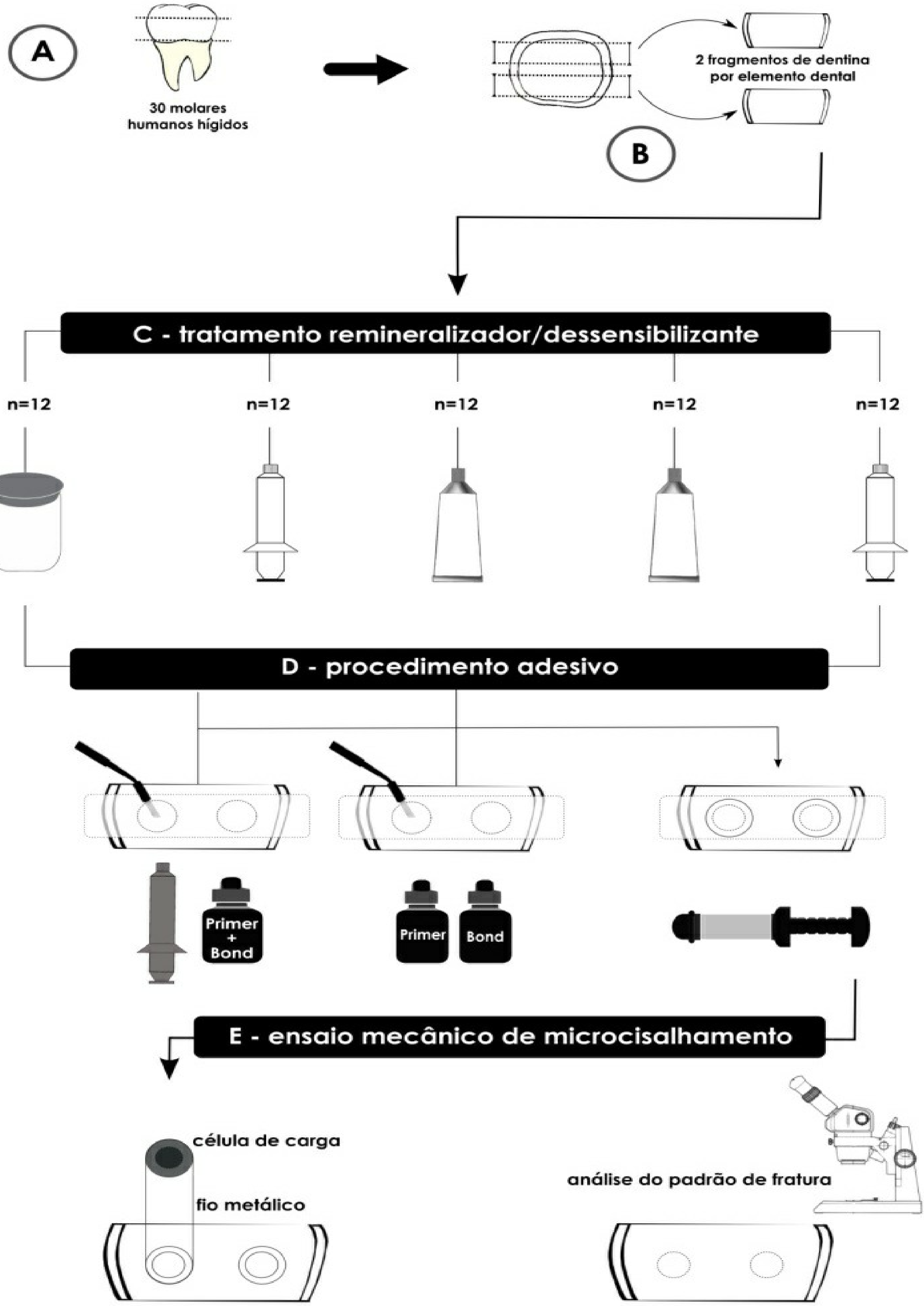

Table 1: Displaying trademark, chemical composition and application protocol

Desensitizing (Trademark and Manufacturer)	**Chemical Composition**	**Application Protocol**
Potassium oxalate (Oxa-gel, Art-Dent/Kota Import ' s, SP, Brazil)	oxalate- Monopotassium-monohydrogenated 3%, carbometilcelulose (pH=4)	Application of the product on the dentin surface with the aid of a brush, for 2 minutes. Remove excess gel.
Recaldent™ + fluoride (MI Paste Plus™, GC Corporation, Tokio, Japan)	Pure water, glycerol, CPP-ACP (Recaldent ™), D-Sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavouring, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, guar gum, propyl p-hydroxybenzoate, butyl hydroxybenzoate, sodium fluoride 0.2 p/p (900 ppm F)	Apply the product to tooth surface. Leave for at least 5 minutes. Do not wash, letting the excess slowly dissolve.
Pró-Argin™ (Sensitive Pró-Alivio, Colgate Oral Pharmaceuticals, PA, USA)	Arginine 8%, hydrated silica, calcium carbonate, glycerin, water, bicarbonate, sodium carboxymethyl cellulose, fragrance, sodium saccharin, blue 1.	Apply the product on the tooth surface during 3 seconds with rubber Cup. Repeat the procedure.
Nano-hidroxiapatite (Nano P, FGM ind, SC, Brasil)	Nanometer calcium phosphate (in the form of hydroxyapatite), sodium fluoride, potassium nitrate, distilled water, thickener, surfactant, humectant, aroma, sweetener and preservative.	Rub the product onto the tooth surface during 10 seconds with rubber Cup. Leave the material to stand for 5 minutes and then remove the excess with a dry cotton roll or lightly moistened.

Table 2: Adhesive systems evaluated, chemical composition and application protocol

Adhesive System (MANUFACTURER)	**Chemical Composition**	**Application Protocol**
Adper Single Bond 2 (3M ESPE)	Bis-GMA, HEMA, diurethane dimethacrylate, polyalkenoic acid copolymer, camphorquinone, water, ethanol and glycerol 1.3 dimethacrylate, 10% by weight of silica nanoparticles (5 nm).	Dentin conditioning with H_3PO_4 35% for 10 s; washing for 10 s; with mild air jet drying; apply 2 consecutive layers of adhesive with brush saturated with material stirring gently on the surface for 15 seconds. Dry gently to evaporate the solvent. Light cure for 10 seconds.
Clearfil SE Bond (Kuraray Co. Ltd.)	*Primer*: 10-MDP; HEMA; hydrophilic dimethacrylate; camphorquinone; aromatic tertiary amine;H_2O *Bond*: 10-MDP; Bis-GMA; HEMA; hydrophobic dimethacrylate; camphorquinone; aromatic tertiary amine; Colloidal silanized silica	Apply the primer for 20 s; dry with air jet; apply the Bond; apply light air jet; light cure for 10 s.

Table 3: Test results micro-shear relating to mechanical treatments desensitizers and adhesive systems

Desensitizing Treatment	**Adhesive System**	
	Etch and rinse	**Self-etching**
Control - without treatment	24,31 (5,17)^{aA}	28,93 (2,61)^{AA}
Potassium oxalate	18,56 (4,41)^{bA}	18,86 (6,18)^{BA}
Arginine	16,69 (3,44)^{bA}	6,55 (6,24)^{cB}
CPP-ACP	30,97 (2,58)^{cA}	30,49 (3,67)^{AA}
Nano-hidroxiapatite	19,94 (3,80)^{bA}	28,50 (3,32)^{aB}

Different superscript lowercase letters indicate significant difference among treatments for the same bonding system. Different superscript letters indicate statistical differences between different adhesive systems to the same desensitizing treatment (p > 0.05).

Figure 2: Results in MPa, desensitizing agents control groups and the two self-etching adhesives and wash and condition

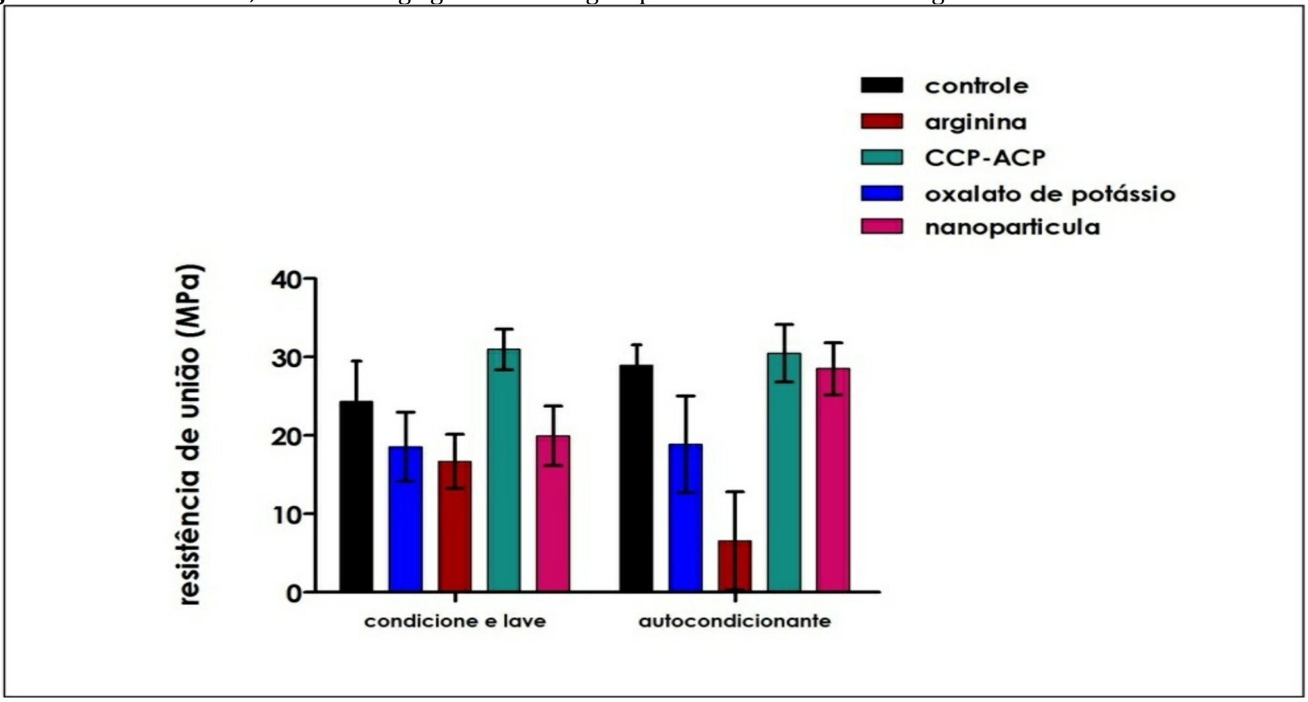

Chart 1: Percentage of the types of fractures of desensitizing substances control group and the stickers self-etching primer and etch and rinse